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Preserved Autonomic Cardiovascular Regulation With Cardiac Pacemaker Inhibition: A Crossover Trial Using High-Fidelity Cardiovascular Phenotyping

Karsten Heusser, MD; Jens Tank, MD; Julia Brinkmann, MD; Christoph Schroeder, MD; Marcus May, MD; Anika Großhennig, PhD; Daniela Wenzel, PhD; André Diedrich, MD; Fred C. G. J. Sweep, MD, PhD; Heidrun Mehling, MD; Friedrich C. Luft, MD; Jens Jordan, MD

Background—Sympathetic and parasympathetic influences on heart rate (HR), which are governed by baroreflex mechanisms, are integrated at the cardiac sinus node through hyperpolarization-activated cyclic nucleotide-gated channels (HCN4). We hypothesized that HCN4 blockade with ivabradine selectively attenuates HR and baroreflex HR regulation, leaving baroreflex control of muscle sympathetic nerve activity intact.

Methods and Results—We treated 21 healthy men with 2×7.5 mg ivabradine or placebo in a randomized crossover fashion. We recorded electrocardiogram, blood pressure, and muscle sympathetic nerve activity at rest and during pharmacological baroreflex testing. Ivabradine reduced normalized HR from 65.9 ± 8.1 to 58.4 ± 6.2 beats per minute ($P < 0.001$) with unaffected blood pressure and muscle sympathetic nerve activity. On ivabradine, cardiac and sympathetic baroreflex gains and blood pressure responses to vasoactive drugs were unchanged. Ivabradine aggravated bradycardia during baroreflex loading.

Conclusions—HCN4 blockade with ivabradine reduced HR, leaving physiological regulation of HR and muscle sympathetic nerve activity as well as baroreflex blood pressure buffering intact. Ivabradine could aggravate bradycardia during parasympathetic activation.

Clinical Trial Registration—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT00865917. (*J Am Heart Assoc.* 2016;5:e002674 doi: 10.1161/JAHA.115.002674)

Key Words: autonomic nervous system • baroreflex control • funny channel • heart rate • hemodynamics • inhibitor • microneurography • pharmacology • physiology • sinoatrial node

Medication effects on human cardiovascular regulation are difficult to predict based on preclinical and clinical investigations. Drugs could interfere with cardiovascular regulation in brain or periphery directly or through secondary baroreflex-mediated adjustments in heart rate (HR) and

vascular tone. High-fidelity phenotyping during infusion of vasoactive medications can be used to detect drug effects on human cardiovascular control that could otherwise go undetected. Ivabradine inhibits hyperpolarization-activated cyclic nucleotide-gated potassium channel 4 (HCN4). HCN4 generates “funny” pacemaker f currents (I_f) promoting slow diastolic depolarization and cardiac rhythm generation.¹ Intracellular cyclic adenosine monophosphate (cAMP) modulates I_f ,² and β -adrenergic stimulation augments cAMP, thereby increasing HCN4 conductivity and HR. Cholinergic stimulation elicits the opposite response. Given HCN4’s important role in autonomic HR adjustments, pharmacological HCN4 inhibition could profoundly affect human cardiovascular regulation. In fact, chronotropic competence is reduced³ or absent⁴ in mutated HCN4 channels unresponsive to cAMP. Conversely, blockade of HCN4 channels on baroreceptor sensory neurons increased their excitability,^{5,6} which would tend to facilitate afferent baroreflex signaling and baroreflex function. The issue is relevant because ivabradine^{7,8} is used in the treatment of heart failure⁹ and angina pectoris¹⁰; however, the influences of HCN4 on human cardiovascular autonomic regulation have not been

From the Institutes of Clinical Pharmacology (K.H., J.T., J.B., C.S., M.M., J.J.) and Biostatistics (A.G., D.W.), Hannover Medical School, Hannover, Germany; Division of Clinical Pharmacology, Department of Medicine, Autonomic Dysfunction Service, Vanderbilt University, Nashville, TN (A.D.); Department of Laboratory Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands (F.C.G.J.S.); Experimental Clinical Research Center, Charité Medical Faculty and Max Delbrück Center for Molecular Medicine, Berlin, Germany (H.M., F.C.L.).

Correspondence to: Jens Jordan, MD, Institute of Clinical Pharmacology, Hannover Medical School, Carl Neuberg Str. 1, 30625 Hannover, Germany. E-mail: jordan.jens@mh-hannover.de

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studied, aside from noninvasive baroreflex assessment of profound sympathetic activation.¹¹ Ivabradine is an open-channel blocker¹² producing “frequency-dependent” or “use-dependent” HCN4 inhibition,¹³ thus ivabradine may be more effective at increased HR.¹⁴ We tested the hypothesis that pharmacological HCN4 blockade with ivabradine affects baroreflex HR regulation in healthy participants such that HR is reduced at a given blood pressure (BP). Moreover, we assessed whether ivabradine would interfere with baroreflex regulation of HR and sympathetic efferent traffic to resistance vessels and whether or not baroreflex BP buffering is perturbed. Finally, we evaluated whether or not in vivo responses mirror ivabradine’s use-dependent pharmacology.

Methods

Participants

Healthy men (aged 18–40 years) with a body mass index between 18 and 30 kg/m² and a resting HR ≥ 60 beats per minute (bpm) were eligible for this study. Preexisting diseases were ruled out through a detailed history, medical examination, 12-lead ECG, BP recordings, and blood sampling for routine laboratory measurements. All procedures were in accordance with the Helsinki Declaration of 1975 (as revised in 1983). A national competent authority and the local internal review board approved the studies. Written informed consent was obtained before inclusion.

Study Design and Protocol

This randomized, double-blind, 2-period, 2-sequence, crossover study was conducted at 2 sites (Experimental Clinical Research Center, Charité Medical University, Berlin, Germany, and Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany). Randomization for study drug sequence (randomization block size: 6) and manufacturing and labeling of blinded medications was done centrally by the pharmacy of the Charité Medical University. Medications were prepared as identical capsules in neutral containers labeled with the randomization code and a visit identifier. After inclusion, participants were consecutively allocated to the next available randomization number. Adherence to randomized medication sequence was secured by following the predefined numeric sequence of the visits. Consequently, participants and investigators remained fully blinded until the database had been locked, double checked, and transferred to the Institute of Biostatistics at Hannover Medical School. The study was registered at ClinicalTrials.gov (identifier NCT00865917).

On 2 separate occasions, participants ingested maximally recommended doses of ivabradine (7.5 mg) or matching placebo 13 hours and 1 hour before testing. The washout

period between study days was at least 3 weeks. Testing was conducted between 8 and 11 AM in a quiet laboratory at an ambient temperature of 22 to 23°C.

An ECG was continuously recorded (Niccomo, Medis GmbH) for HR determination. Recumbent systolic BP (SBP), mean BP, and diastolic arterial BP were measured with an automated oscillometric device (Dinamap; GE/Critikon). We obtained blood samples after at least 30 minutes of rest for plasma catecholamine determination with high-pressure liquid chromatography and consecutive electrochemical detection.¹⁵ Stroke volume and cardiac output were obtained using an inert gas rebreathing technique (Innocor; Innovision). Muscle sympathetic nerve activity (MSNA) was recorded from the right peroneal nerve (Nerve Traffic Analyzer 662C-3; Biomedical Engineering Department, University of Iowa, Iowa City, IA), as described previously.¹⁶ Data were analog-to-digital converted and analyzed using a program written by one of the authors (A.D.). We determined the following MSNA parameters from the integrated nerve signal: burst frequency, or the number of MSNA bursts per minute; burst incidence, or the number of bursts per 100 heart beats; and total activity, or the area under the bursts per minute as arbitrary units per minute.

Following a resting period of at least 30 minutes, we obtained resting baseline recordings in the supine position. After taking blood samples and measuring cardiac output, we performed a pharmacological baroreflex assessment using the nitric oxide donor sodium nitroprusside (vasodilator) and the $\alpha 1$ -adrenoceptor agonist phenylephrine (vasoconstrictor). Incremental infusions were given over periods of 6 minutes per dose step. Dose incrementation was stopped after the maximum dose of 2.1 $\mu\text{g/kg}$ per minute had been reached, SBP had changed by >25 mm Hg, or HR had dropped <40 bpm.

End Points

Main end points of the study were normalized HR (ie, HR at a given BP) and resting MSNA burst frequency during supine rest. Exploratory end points served to further characterize hemodynamic and autonomic responses to ivabradine at rest and during pharmacologic baroreflex challenge: arterial BP, stroke volume, cardiac output, total peripheral resistance, plasma catecholamine levels, cardiac and sympathetic baroreflex characteristics, and vasoactor sensitivity. The latter was defined as SBP change per dose increment of sodium nitroprusside and phenylephrine, respectively.

HR Normalization

Ivabradine is expected to change HR directly, but it may do so also indirectly via baroreflexes through changes in BP. To remove confounding baroreflex influences on resting HR, we normalized HR before data unblinding (example in Figure 1):

We transferred the relationship between RR intervals (RRIs) and corresponding SBP values during pharmacological baroreflex testing into a mathematical function by linear regression or baroreflex curve fitting using a Boltzmann sigmoidal function separately for the placebo and ivabradine condition for each participant (sigmoidal curves in the example). Resting SBP of both visits (vertical dotted lines in the Figure 1) were averaged. The average served as shared common (standard) pressure (vertical solid line in the figure). This standard pressure was used as input (abscissa value) to the baroreflex curve function to calculate normalized RRI and HR for each visit separately (ordinate values).

Merged Baroreflex Curves

Baroreflex-mediated changes in the dependent parameters (MSNA, RRI) were collected in 10-mm Hg bins separately for placebo and ivabradine. Related bins for all participants were merged. Using GraphPad Prism 5, a Boltzmann sigmoidal function was fitted to these merged data, and 95% confidence bands were calculated and plotted. Note that the bins for lowest and highest BP are least occupied by values, thus their weight in calculations of curve-fitting parameters and confidence bands are small (Figure 6A and 6B).

Statistics

We expected to observe an ivabradine-related HR reduction by ≈ 6 bpm. With SD of 6.7 bpm in the group's resting HR paired differences, $\alpha=0.05$, and 2-sided testing, 15 participants would

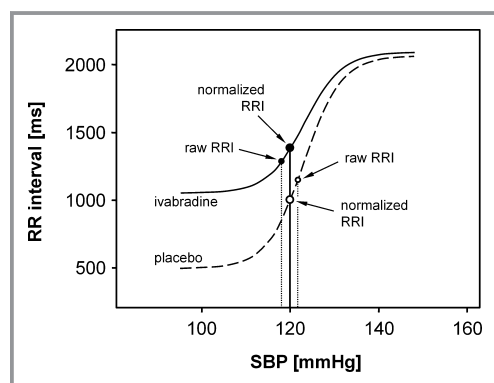


Figure 1. HR normalization procedure. The example assumes lower SBP on ivabradine than on placebo (118 vs 122 mm Hg). Related RRIs are labeled “raw RRI.” The solid vertical line represents mean SBP (120 mm Hg), which determines “normalized RRI” values. Without normalization, ivabradine’s effect on HR would have been underestimated, as demonstrated by the smaller difference between the 2 raw RRI values compared with the normalized RRI values. HR indicates heart rate; RRI, RR interval; SBP, systolic blood pressure.

provide $\geq 80\%$ statistical power to detect such a difference.¹⁷ We included additional participants to allow for the meaningful analysis of secondary and exploratory end points. According to a prospective data analysis plan, primary and secondary end points were analyzed using the Hills-Armitage approach.¹⁸ The method allows period-adjusted therapy effect estimation, namely, calculation of period-adjusted *P* values for mean differences between placebo and ivabradine data. To appreciate consistency of the results, several sensitivity analyses were performed (univariate 1-sample *t* tests and mixed-model analysis with participant as random effect). Exploratory variables have been tested with *t* tests for paired data and correlation analysis without adjustments for multiple testing. A value of $P<0.05$ was considered significant. If not otherwise indicated, data are expressed as mean \pm SD.

Results

We screened 26 men: 23 met inclusion and exclusion criteria and entered the study. Twenty-one men completed both study

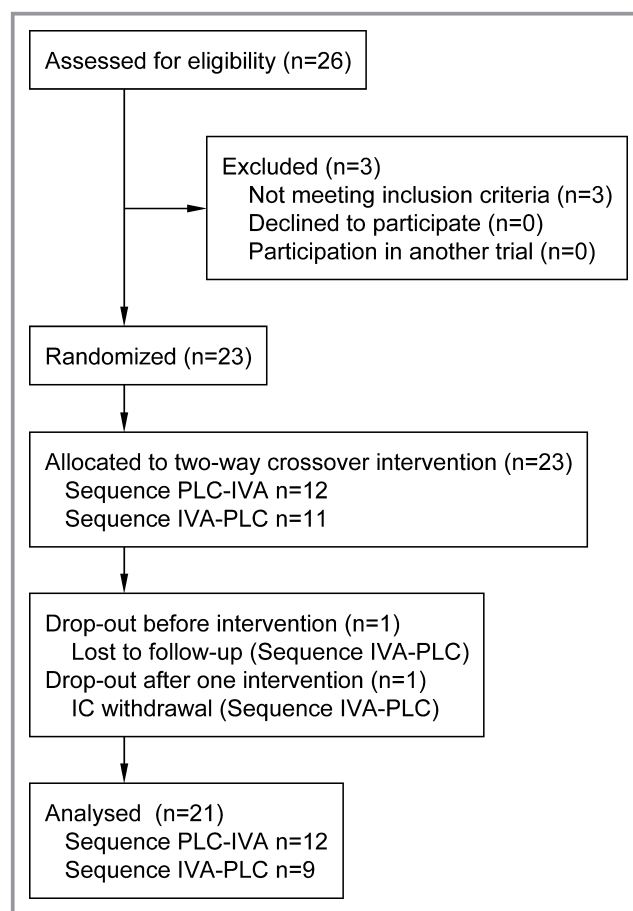


Figure 2. CONSORT flow diagram of participant enrollment, allocation, and analysis. IC indicates informed consent; IVA, ivabradine; PLC, placebo

visits (2 dropped out) (Figure 2). Microneurographic recordings were obtained in 18 participants on both days. In 3 participants, we failed to obtain a stable nerve recording position at the second experimental day. The characteristics of the participants were as follows: age 26.8 ± 4.2 years, height 1.82 ± 0.06 m, body mass 79.8 ± 10.5 kg, body mass index 24.1 ± 3.0 kg/m², BP $130 \pm 8/72 \pm 8$ mm Hg, and HR 67.4 ± 5.8 bpm. Two adverse events (insomnia, hyperhidrosis) occurred during the study. The events were not judged to be related to the study medication. There were no serious adverse events.

Experimental data are shown in Table. I_f blockade reduced resting HR with and without normalization for prevailing BP (Figure 3A) (for HR normalization, see Methods). Ivabradine did not influence resting MSNA (Figure 4). BP and stroke volume remained unchanged with a trend toward reduced cardiac output ($\approx 7.6\%$) and increased total peripheral resistance ($\approx 5.6\%$) on ivabradine. Plasma cate-

cholamine levels were similar under both conditions. SBP sensitivity to sodium nitroprusside infusion was similar on both study days with a trend toward reduced sensitivity (improved BP buffering) to the vasoconstrictor phenylephrine with I_f blockade (Figure 5). Ivabradine did not alter pharmacological baroreflex sensitivities, such as the cardiac parasympathetic and vasoconstrictor sympathetic baroreflex gains. I_f blockade dampens the increase in MSNA burst frequency during higher sodium nitroprusside infusion rates, namely, when high vasoconstrictor activity and elevated HR co-occur (Figure 6A); however, HR-independent MSNA parameters (ie, MSNA burst incidence and total activity) were not altered by HCN4 blockade during pharmacological baroreflex testing (data not shown). Ivabradine caused a “parallel” upward shift of the cardiac baroreflex curve to longer RRIs (Figure 6B). Consequently, RRI lengthening by ivabradine was virtually independent of the prevailing HR during pharmacological baroreflex testing.

Table. Hemodynamic, Sympathetic, Hormonal, and Baroreflex Parameters

Parameter	Placebo	Ivabradine	P Value
Hemodynamics			
HR, bpm	65.9 \pm 8.1	58.4 \pm 6.2	<0.001
HR _p , bpm	52.7 \pm 7.4	47.3 \pm 5.5	<0.002
SBP, mm Hg	123 \pm 8	122 \pm 8	0.649
MBP, mm Hg	89 \pm 4	89 \pm 4	0.894
DBP, mm Hg	68 \pm 5	69 \pm 5	0.582
CO, L/min	7.74 \pm 1.60	7.19 \pm 1.15	0.071
SV, mL	118 \pm 23	118 \pm 18	0.899
TPR, dyn-s/cm ⁵	956 \pm 185	1010 \pm 160	0.088
Sympathetic activity (MSNA bursts)			
Frequency, bursts/min	28.4 \pm 7.9	26.2 \pm 6.7	0.221
Incidence, bursts/100 heart beats	46.0 \pm 11.3	45.3 \pm 12.1	0.575
Total activity, au	1.14 \pm 0.45	1.26 \pm 0.76	0.892
Hormones			
Dopamine, pmol/L	69 \pm 25	70 \pm 14	0.520
Norepinephrine, pmol/L	1040 \pm 500	910 \pm 210	0.654
Epinephrine, pmol/L	135 \pm 84	132 \pm 64	0.553
Pharmacological baroreflex and vasoactor sensitivity			
Cardiac BRG, ms/mm Hg	16.8 \pm 8.4	14.8 \pm 6.5	0.151
Sympathetic BRG, bursts/min/mm Hg	-2.74 \pm 1.07	-2.53 \pm 0.83	0.247
NTP sensitivity, mm Hg μ g ⁻¹ min ⁻¹ kg ⁻¹	-7.9 \pm 6.4	-6.2 \pm 4.6	0.158
PHE sensitivity, mm Hg μ g ⁻¹ min ⁻¹ kg ⁻¹	20.5 \pm 8.0	18.0 \pm 6.3	0.073

au indicates arbitrary units; BRG, baroreflex gain (ie, baroreflex sensitivity); CO, cardiac output; DBP, diastolic blood pressure; HR, normalized (baroreflex-corrected) heart rate; HR_p, normalized heart rate during parasympathetic activation; MBP, mean blood pressure; MSNA, muscle sympathetic nerve activity; NTP, sodium nitroprusside; PHE, phenylephrine; SBP, systolic blood pressure; SV, stroke volume; TPR, total peripheral resistance.

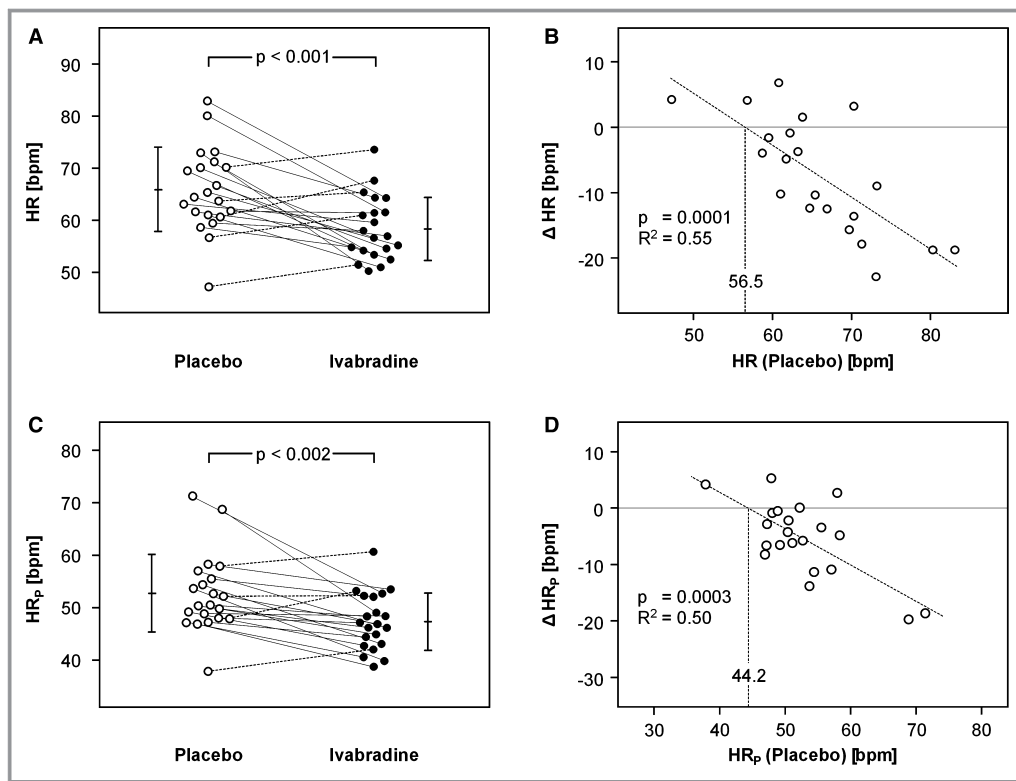


Figure 3. Individual effects of HCN4 inhibition on HR. A, Effects of HCN4 inhibition on resting HR. Solid and dashed lines denote HR decreases and increases, respectively. B, Ivabradine's use dependence: HR lowering is more pronounced in participants with higher resting HR. With resting values of ≈ 56.5 bpm, ivabradine has no effect (unresponsive HR). C, Effects of HCN4 inhibition on HR during parasympathetic activation (HR_p) through baroreflex loading with phenylephrine. HR_p was assessed as HR at the largest individual phenylephrine dose that was reached on both study days. Solid and dashed lines denote HR_p decreases and increases, respectively. D, Participants with lower HR_p on placebo exhibited a smaller reduction in HR_p with ivabradine. The observation is consistent with ivabradine's use-dependent pharmacology; however, parasympathetic activation shifted the unresponsive HR to ≈ 44.2 bpm, which is ≈ 12 bpm lower compared with resting conditions (see panel B). bpm indicates beats per minute; HR, heart rate.

Participants with higher resting HR on placebo exhibited more pronounced HR slowing on ivabradine, showing "use dependence" (Figure 3B). Participants with HR below ≈ 56.5 bpm did not respond to I_f blockade. We observed a similar pattern during baroreflex loading with phenylephrine (Figure 3C and 3D); however, the HR at which ivabradine did not slow HR further was only ≈ 44.2 bpm (ie, ≈ 12 bpm lower during parasympathetic activation). Together, Figure 3B and 3D visualize ivabradine's use-dependent characteristics; however, they also show, as does Figure 6B, that ivabradine and parasympathetic activation are additive in terms of HR reduction.

Discussion

We observed that HCN4 blockade with ivabradine reduces HR, leaving baroreflex HR and MSNA regulation intact. Although HCN4 is involved in transducing autonomic

activity at the sinus node level, baroreflex HR regulation was preserved on ivabradine. The cardiac baroreflex curve was parallel-shifted toward lower HR without reductions in baroreflex gain or range. Similarly, baroreflex HR slopes determined noninvasively by cross-spectral analysis or the sequence method were unchanged with ivabradine in a human model of cardiac sympathetic activation.¹¹ Because MSNA discharges occur during diastole when arterial baroreceptors are unloaded, we expected reduced MSNA frequencies with compensatory increases in sympathetic action potentials within bursts; however, ivabradine-induced HR reduction was not associated with resting MSNA changes. Moreover, sympathetic baroreflex regulation was virtually identical on placebo and on ivabradine considering HR-independent MSNA measurements. Finally, BP responses to vasoactive medications did not increase on ivabradine, suggesting that baroreflex BP buffering remained fully functional.¹⁹

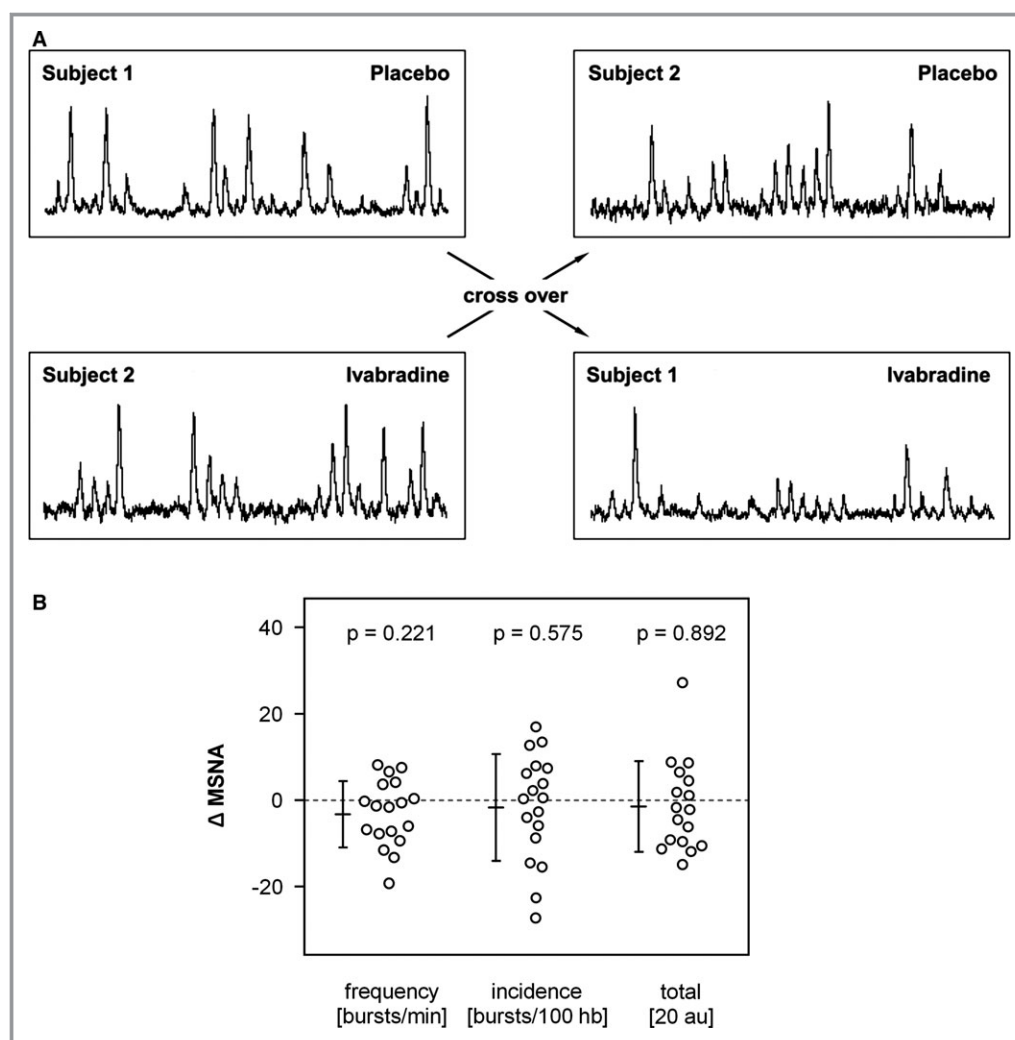


Figure 4. Effects of HCN4 inhibition on MSNA. A, Original recordings of 2 participants. B, Individual changes in MSNA burst frequency, burst incidence, and total MSNA by ivabradine compared with placebo. Total MSNA has been rescaled ($\times 20$) for visual range harmonization among the 3 MSNA measures (also see Table). au indicates arbitrary units; hb, heart beats; MSNA, muscle sympathetic nerve activity.

Together with previous studies in genetic animal models and in patients with familial sinus bradycardia due to HCN4 mutations,^{3,20–23} our study provides new insight into HCN4's role in HR regulation. In patients with the 573X mutation, HCN4 does not respond to cAMP.³ Nevertheless, HR increased ≈ 50 bpm during exercise. Likewise, HR acceleration to β -adrenoreceptor stimulation was intact in adult HCN4-deleted mice²⁴ and in a heterozygous knock-in model rendering HCN4 cAMP insensitive.⁴ On ivabradine, chronotropic competence in terms of HR acceleration and deceleration over a wide HR range assessed through baroreflex loading and unloading was unchanged. Consequently, I_f cannot be the crucial mechanism transducing autonomic signals at the level of the sinus node.

Cardiac pacemaker models are composed of several clocks,^{25,26} raising questions about HCN4's dominant role in sinoatrial node automatism and rate regulation. Instead,

HCN4 may defend the sinus node against excess hyperpolarization,²⁷ preventing bradycardia. Despite its use-dependent pharmacology,¹² indicated by larger HR reduction in persons with higher resting HR, ivabradine aggravated baroreflex-mediated bradycardia in our study. Moreover, in the SIGNIFY study, almost 10 000 patients with stable coronary artery disease received ivabradine doses up to 10 mg twice daily. Bradycardia and atrial fibrillation occurred more often in the ivabradine group.²⁸ Together with our mechanism-oriented study, these observations translate the preclinical concept that I_f is a defense mechanism against excess bradycardia to humans. Using a similar approach, we previously observed that α_2 -adrenoreceptor stimulation with clonidine also disables physiological safeguards maintaining HR.²⁹

Our study has potential limitations. We did not investigate responses to ivabradine under steady state conditions;

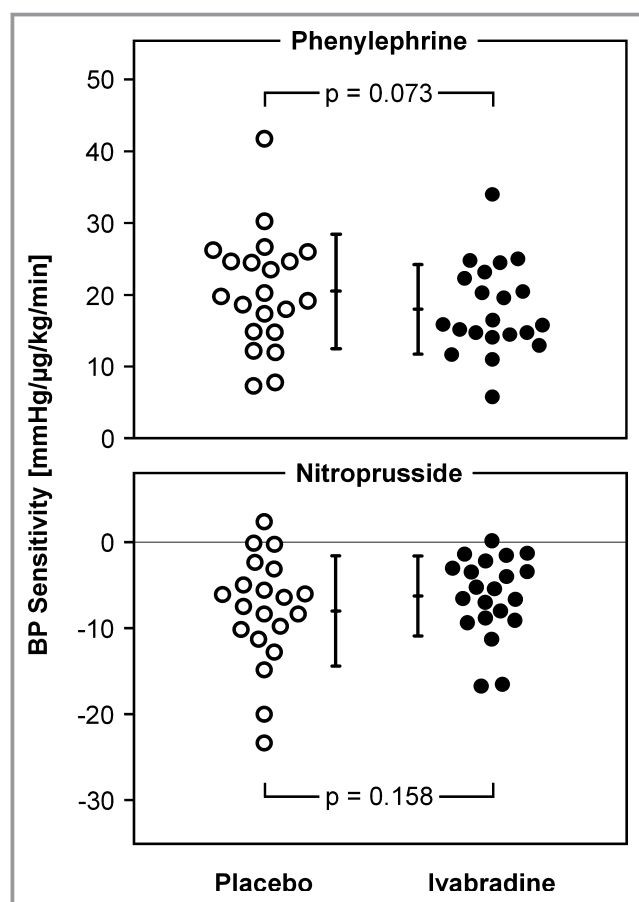


Figure 5. BP sensitivity to vasoactors. Effects of HCN4 inhibition on BP sensitivity to infusion of the vasoconstrictor phenylephrine (upper panel) and the vasodilator sodium nitroprusside (lower panel) during placebo (open circles) and ivabradine (closed circles). Note the trend toward improved baroreflex buffering of BP increases on ivabradine (upper panel). BP indicates blood pressure.

however, we showed earlier that acute dosing 13 hours and 1 hour before measurements was sufficient to attain ivabradine plasma concentrations in a therapeutic range.¹¹ Furthermore, the drug's bradycardic activity consists of an initial rapid and subsequent relatively long duration of action caused by the N-dealkylated metabolite and the parent drug, respectively.³⁰ On the other hand, the 3-week washout period was more than twice the time required to avoid carryover, according to the kinetics of the bradycardic effect with repeated oral administration.³⁰ Ivabradine's pharmacological interaction with HCN4 channels complicates the interpretation of our study. As an open-channel blocker, for example, ivabradine features use dependence with trapping of the drug in the closed HCN4 channel.⁸ Furthermore, HCN4 is also expressed in the nervous system, particularly in the thalamus,³¹ in spinal interneurons,³² and in baroreflex afferents.^{5,6} MSNA is a sensitive

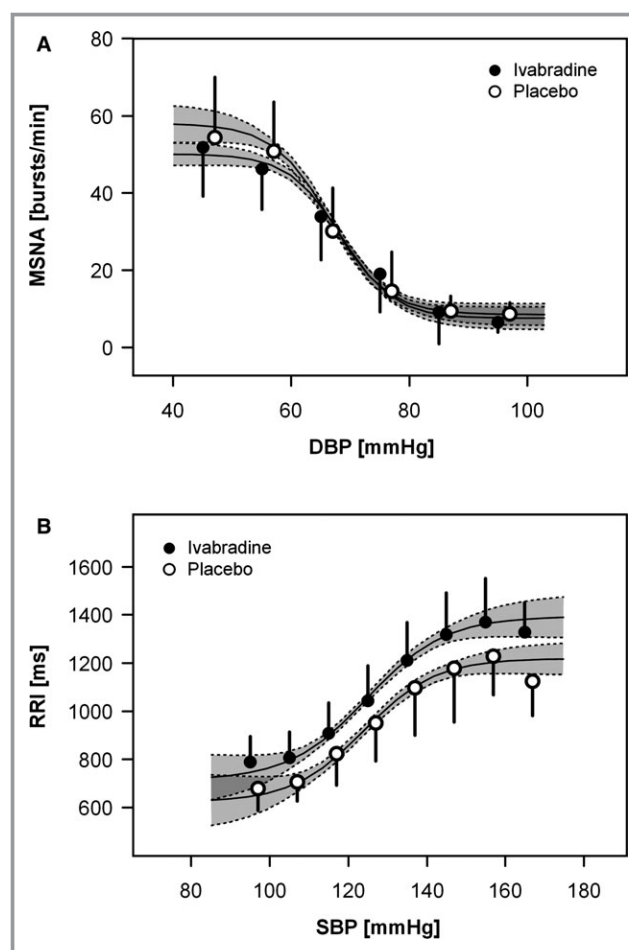


Figure 6. Interactions between ivabradine and arterial baroreflexes. A, Sympathetic baroreflex: Although ivabradine may dampen the increase in MSNA burst frequency during nitroprusside infusion (ie, when high vasoconstrictor activity and elevated HR co-occur), baroreflex curves are virtually identical if HR-independent MSNA measures (burst incidence and total activity) are used (curves not shown). B, Cardiac baroreflex: Ivabradine shifts ("offsets") the baroreflex curve upward (to longer RRIs) with virtually no effect on slope or range. Shaded areas indicate 95% confidence bands. Note that the bands do not overlap with high blood pressure, indicating that baroreflex-mediated HR slowing is intensified by ivabradine. DBP indicates diastolic blood pressure; HR, heart rate; MSNA, muscle sympathetic nerve activity; RRI, RR interval; SBP, systolic blood pressure.

readout for changes in central autonomic regulation.^{33–35} Because MSNA did not change, confounding effects by neural HCN4 inhibition is unlikely; however, diseases could alter the ivabradine response. Blunted baroreflex function in type 1 diabetic rats, for example, was associated with increased HCN expression and cAMP sensitivity.⁶ Our findings in healthy participants cannot be simply extrapolated to patients. Finally, given the potential influence of respiration on postganglionic sympathetic discharge, it would have been desirable to also record and analyze

breathing, yet others did not observe effects of ivabradine on respiratory rate either in healthy men³⁶ or in patients with chronic obstructive pulmonary disease.³⁷

Conclusion

High-fidelity physiological phenotyping provides pharmacological insight that could not be gained by routine HR and BP measurements. We observed that HCN4 blockade with ivabradine reduced HR but did not impair sympathetic or parasympathetic baroreflex function. This feature preserves baroreflex-dependent buffering of BP changes and aggravates parasympathetically mediated HR reduction; therefore, ivabradine's use dependence is not protective against marked bradycardia. Our results could explain unfavorable responses to ivabradine in recent clinical trials. Moreover, our findings provide a better understanding of HCN4's physiology in humans, which could be applied to elucidate diseases associated with perturbed HR regulation. Excess HCN4 conductivity, for example, could contribute to postural tachycardia syndrome, a condition characterized by sinus tachycardia with standing and a mismatch between cardiac and vascular sympathetic activation.^{38,39} Indeed, HCN4 inhibition may improve symptoms of postural tachycardia syndrome.⁴⁰ Although gain-of-function mutations in the *HCN4* gene have not been discovered, functional HCN4 gain of function has been described in right atrial samples from patients with chronic atrial fibrillation.⁴¹ Patients with heart failure or coronary artery disease, who are currently being considered for ivabradine treatment, also show redistribution of sympathetic activity toward the heart with an associated increase in HR.^{42,43}

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Disclosures

None.

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Preserved Autonomic Cardiovascular Regulation With Cardiac Pacemaker Inhibition: A Crossover Trial Using High-Fidelity Cardiovascular Phenotyping

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